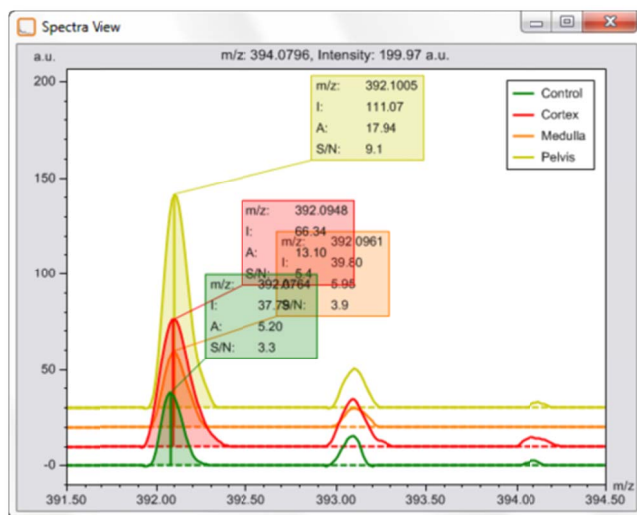
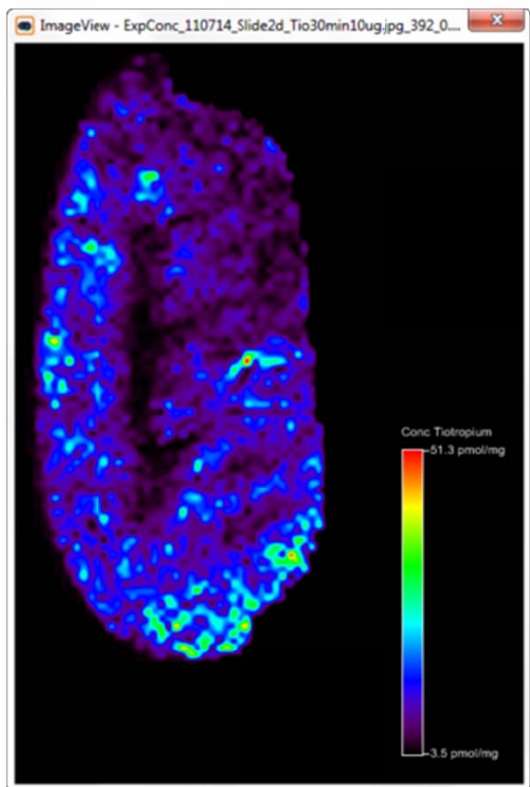
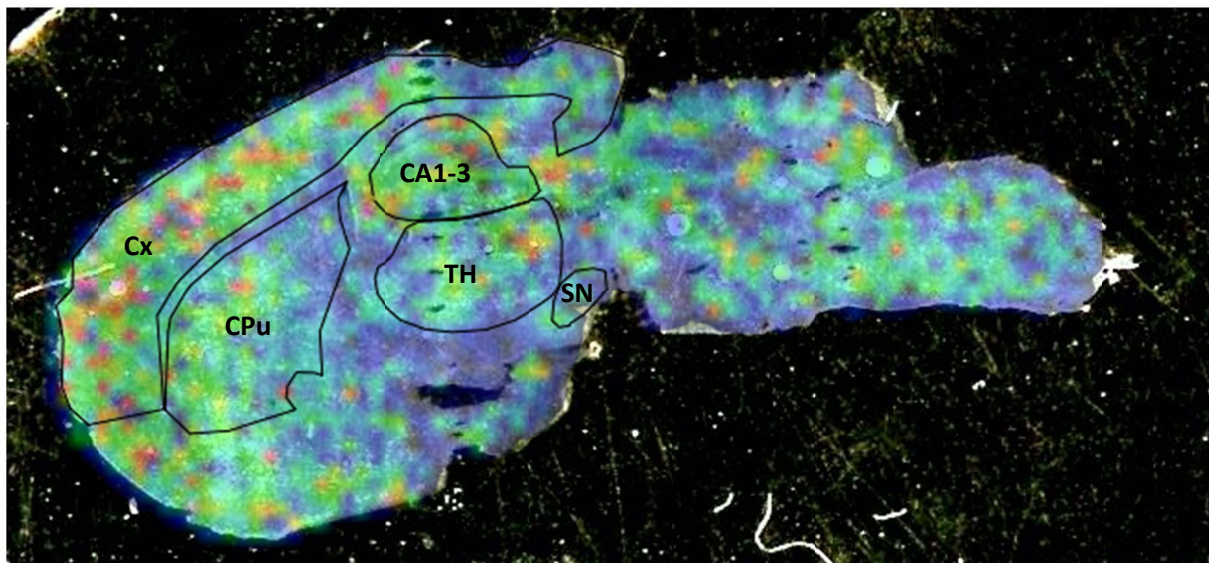


mSIQuant 1.2

User Manual



Evaluation and quantitation tool for MS Imaging

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Legal and Regulatory Notices

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Document History

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Warranty

The information in this document is subject to change without notice.

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License for use and distribution

mslQuant 1.2 is freeware software. mslQuant 1.2 can be used on any computer, including a computer in a commercial organization. mslQuant 1.2 is developed in C++ with Microsoft VS2010, MFC and .NET framework. At present time the source code is not open source.

One doesn't need to register or pay for mslQuant 1.2, but if the software is used for publication, please cite the following article:

Källback P, Shariatgorji M, Nilsson A, Andrén PE. Novel mass spectrometry imaging software assisting labeled normalization and quantitation of drugs and neuropeptides directly in tissue sections.

J Proteomics. 2012 Jul 27. [Epub ahead of print]. [doi:10.1016/j.jprot.2012.07.034](https://doi.org/10.1016/j.jprot.2012.07.034)

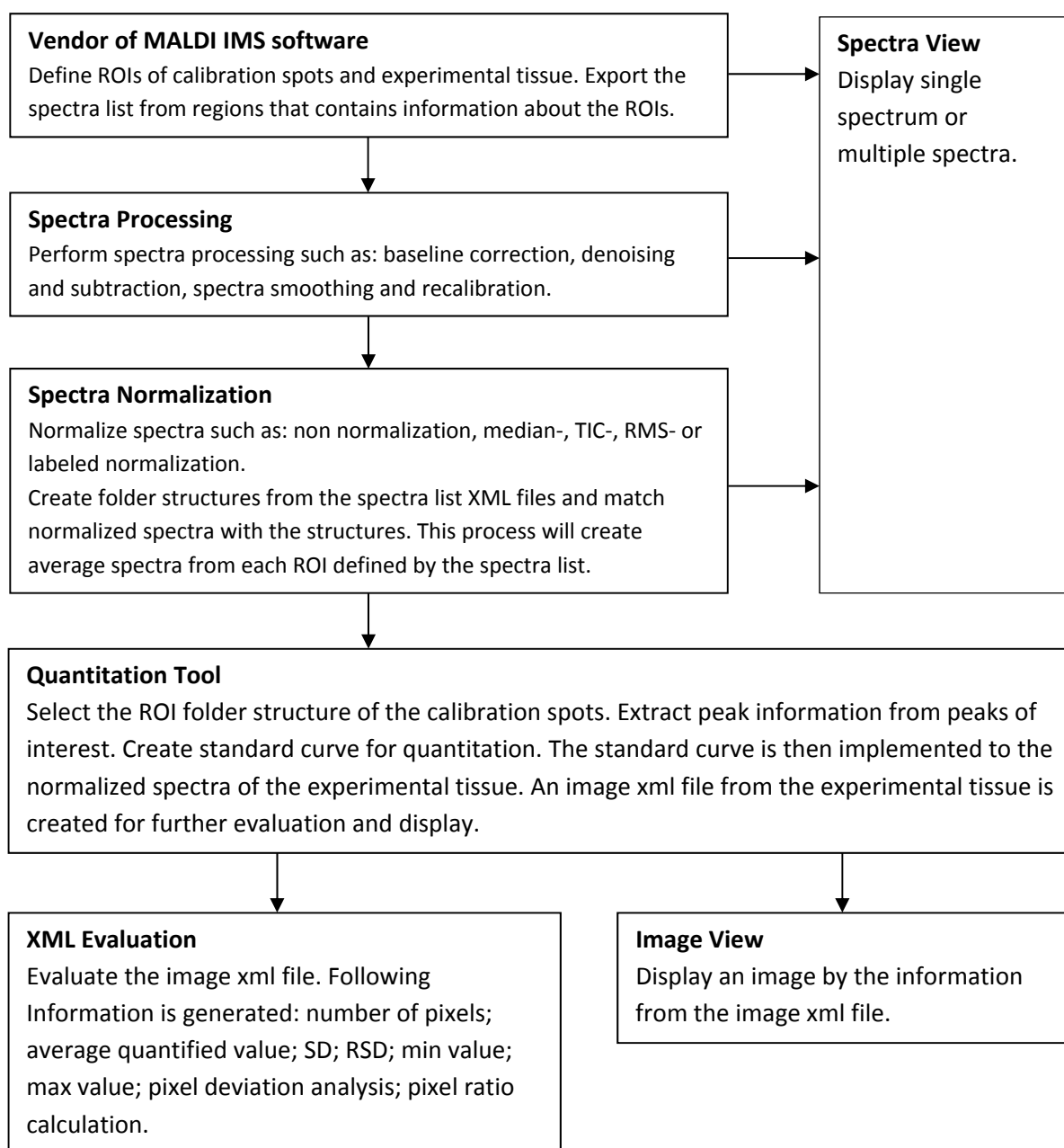
Protocol for Data Analysis

Conditions

Version 1.2 of mslQuant is only working together with Bruker Daltonics flexImaging™ and the data format XMass. If the new Container data format is used it must first be converted with flex Data Converter. Further, only full MS in reflectron or linear mode can be analyzed. LIFT or FAST data can't be analyzed in current version of mslQuant.

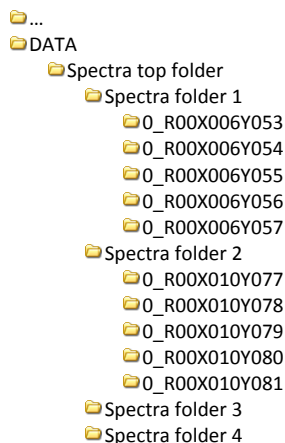
Process flow

The process flow below illustrates the overall protocol for the data analysis:



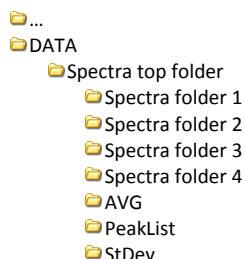
Spectra folder definition

To be able to use the programs in MALDI Tools the user has to understand the overall data structure. There are two different expressions that are used: **spectra top folder** and **spectra folder**. In the spectra folder are all the spectra for regions of interest (ROI) or spectra from different experiments. The spectra top folder though, contains one or several spectra folders. See the structure below.



The manual is also referring to spectrum folder. Spectrum folders are those that are contained in a spectra folder e.g. folder 0_R00X006Y053 is a spectrum folder.

When the data analysis with the program suite in MALDI Tools has been performed there are three new folders in the spectra top folder: AVG, PeakList and StDev folders.



The AVG folder will contain the average spectra for each spectra folder. The StDev folder will contain the standard deviation spectra for each spectra folder and a noise calculation for each average spectra that includes local noise and average spectrum noise. The PeakList folder will contain the peak list for each average spectrum and extracted peak information file for a specific spectrum peak.

Pixel definition

The definition of a pixel in this user manual is the same as sampling position where a mass spectrum is acquired.

Associated files

List of file extensions and their associated programs:

- *.cal – Mass spectrum recalibration file, associated with Spectra Processing
- *.par – Labeled normalization parameter file, associated with Spectra Normalization
- *.dat – Mass spectrum data file, associated with Spectra View

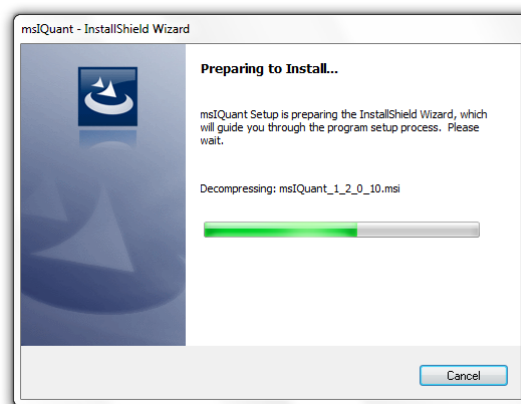
Installation

The installation of mslQuant is very simple and the whole package comes with a single self-extracting file. The installation file can be downloaded at www.maldi.ms.

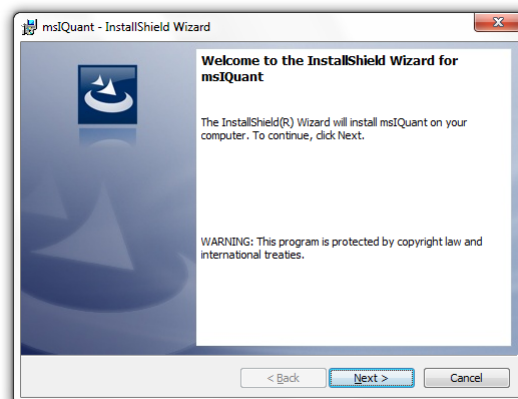
The latest version of the installation is mslQuant 1.2.0.10 and the computer requirement is Windows operating system XP or higher.

After downloading the file (current file name: mslQuant_1_2_0_10.exe) and store it on a place in the computer, double click on the file and it will self-extract. A security warning dialog may appear since no valid digital signature is attached with the installation file.

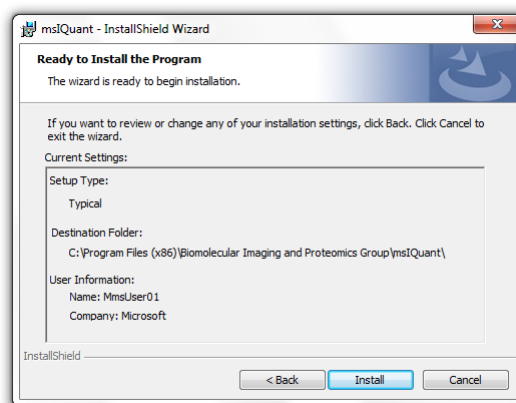
When the self-extracting file starts the installation is prepared.



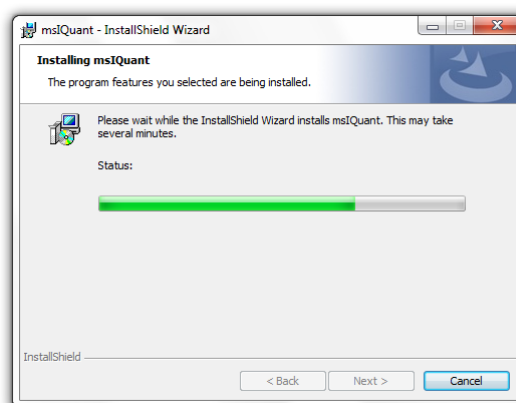
A welcome screen appears. Click the “Next >” button to continue.



Current settings are displayed. Start the installation by click on the “Install” button.



The installation is proceeding.



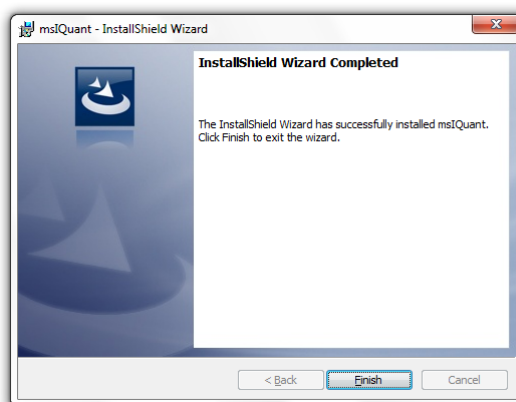
After completed installation, click the “Finished” button. Shortcut icons are displayed on the desktop and in the start menu.

Note: When “Spectra Processing” and “Spectra Normalization” is used for the first time, look for the calibration list and/or the parameter list in the folder:

<C:\Program Files\Biomolecular Imaging and Proteomics Group\ProcessingParameters>

or in 64-bit operating system such as Windows 7:

<C:\Program Files (x86)\Biomolecular Imaging and Proteomics Group\ProcessingParameters>



To uninstall msIQuant go into Add or Remove Programs that is found in the control panel. Search for msIQuant and then uninstall.

Spectra Processing

Spectra Processing is a program that performs general spectra processing. The resulting spectrum is stored in each spectrum folder with a name called <xy.dat> and is located in the same folder hierarchy as the <fid> file. Below is an example of the hierarchy:

```

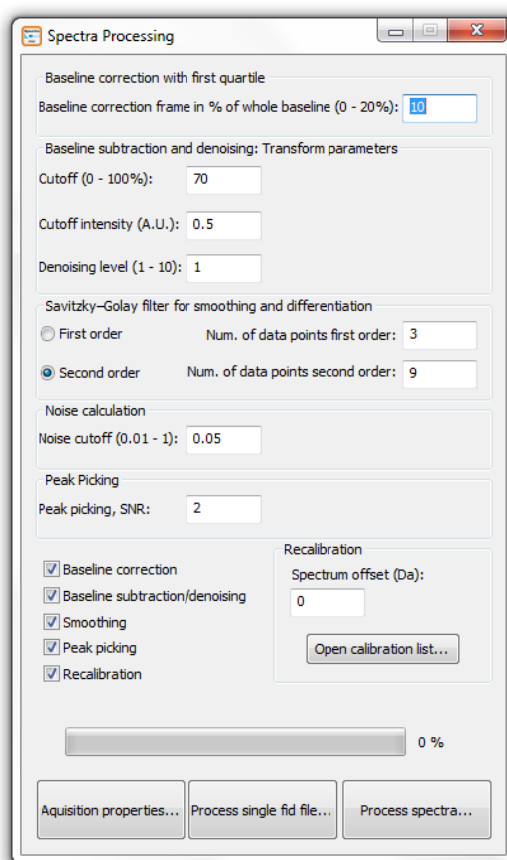
0_R00X011U062
├── 1
│   └── 1Ref
│       ├── pdata
│       ├── acqu
│       ├── acqu
│       ├── fid
│       ├── SpectraProcessing.xml
│       ├── sptype
│       └── xy.dat

```



The spectrum processing steps are:

- Baseline correction
- Baseline subtraction and denoising
- Spectrum smoothing
- Peak picking and Recalibration



Parameters

Baseline correction with first quartile

The baseline correction is an algorithm that straightens out the baseline when the baseline of a spectrum is drifting. The method that is used to create a baseline is the simple moving first quartile (SMQ1). The only parameter to set in this function is the length of the moving frame. The value of this frame can be 0 – 20% of the total m/z channels and the default value is set to 10%. After creation of the baseline the baseline is subtracted with the spectrum. To avoid negative spectrum values the modulus of the largest negative value is added to all intensities for all m/z channels.

Baseline subtraction and baseline denoising: Transform parameters

The baseline subtraction and baseline denoising is performed with an algorithm called sorted mass spectrum transform (SMST). The noise part in the SMST transform is determined by three parameters, Cutoff, Cutoff intensity and Denoising level. If the intensity is higher than the Cutoff intensity at the Cutoff of the SMST transform, the transform is scaled down from 0 to the Cutoff to be equal as the set Cutoff intensity. The peak part of the SMST transform is subtracted by a value so the transform fits the Cutoff intensity. If the intensity is smaller at the Cutoff than the Cutoff intensity no processing is performed. If a more aggressive denoising shall be done the Denoising level is set higher than 1. The derivative at the Cutoff is calculated and is multiplied by the Denoising level. A new Cutoff is determined where this calculated derivative is located and the denoising is performed as described above.

Savitzky-Golay filter for smoothing and differentiation

The spectrum smoothing is done with Savitzky-Golay smoothing filter. The smoothing method can be with either average or 2nd degree polynomial. The frame of data points must also be defined and must consist of an odd number. The default value is set to smoothing of 2nd degree polynomial with a frame of 9 data points.

Noise calculation

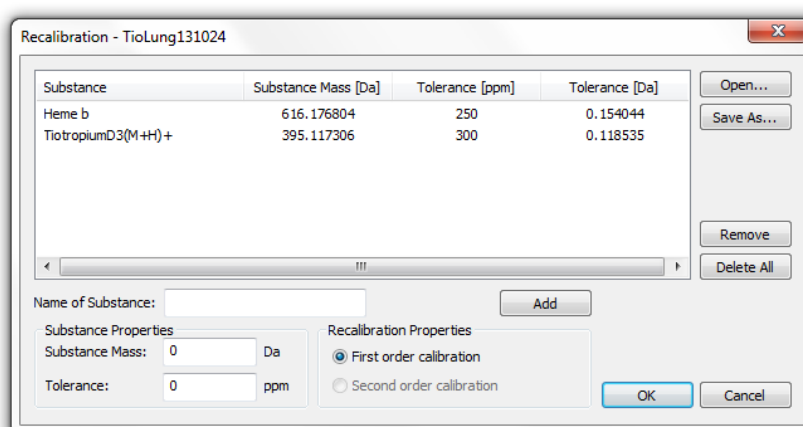
The noise calculation is based on the SMST transform. In this part the SMST transformed is the m/z channels normalized from 0 to 1. The intensity is also normalized from 0 – 1. The transform is then derived. The parameter in the noise calculation, Noise cutoff, is the value of the derivative until the noise is considered to be localized. The default value of the derivative (Noise cutoff) is set to 0.05. All values after this cutoff is set to zero and the SMST is retransformed. The noise is then calculated as the root mean square (RMS) of all peaks in the noise.

Peak Picking

The peak picking algorithm is calculated as the signal to noise ratio (SNR) and the default value is set to 2. The reason to do the peak picking is to be able to do recalibration of the spectrum. The peak picking is done by deriving the spectrum. When the derivative is crossing the x-axis from positive to negative value there might be a potential peak that is determined by the SNR. The peak itself is calculated by applying a 3^d degree polynomial of the spectrum where the derivative is crossing the x-axis

Recalibration

When spectra are acquired during an MALDI image MS experiment they have to be recalibrated afterwards even if a calibration has been performed before an experiment. This is because of how some MALDI MS instrument is constructed. When a time of flight (TOF) MS is used the m/z will differ because of the difference in tissue and matrix thickness. The recalibration is performed against known peaks and the methods to this processing step are single calibrant recalibration, or multiple calibrant recalibrations with 1st or 2nd order. To do a 1st order recalibration at least two known peaks are required. For 2nd order recalibration at least three known peaks are required. For both 1st and 2nd order recalibration a calibration curve is generated with best fit against the calibrant. One point calibration can also be used if only one calibrant is in the list. The one point calibration will only shift the peaks. If a calibration before setting up an experiment has failed, it is possible to correct a spectrum if a mass shift has occurred with up to ± 2 Da. This value is set in the main dialog,



In the recalibration dialog it is possible to open a calibration file that contains the calibrant needed to perform the recalibration. The file extension for the calibration file is `*.cal`. To create a new calibrant list just add the name of the substance in the “Name of Substance” edit window, add the substance mass in Dalton and the tolerance in ppm. Push the “Add” button to add the calibrant in the list. It is possible to edit the list in two ways, delete all substances by clicking the button “Delete All” or delete one substance by first select one and then click the button “Remove”. When the list is created save the list with a unique name by pushing the “Save As...” button. Click the first or second order calibration and click the “OK” button.

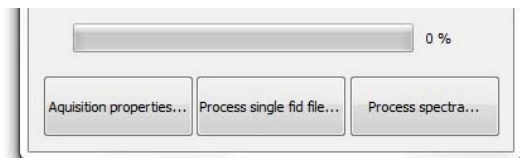
Depending on which spectra processing steps to be done one need to check the different steps to be done. The processing steps that can be selected are:

- Baseline correction
- Baseline subtraction/denoising
- Smoothing
- Peak picking
- Recalibration

Processing

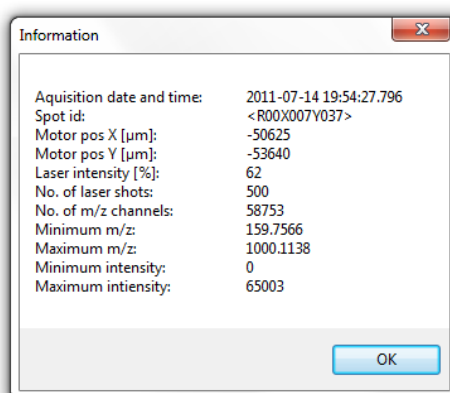
The different processing options are:

- Acquisition properties
- Processing single fid file
- Process spectra



Acquisition properties...

To analyze the properties of a single pixel, click the button “Acquisition properties...” and select the fid file in the spectrum of interest. Below is an example of the information that is put together.



Processing single fid file...

To process a single spectrum, click the button “Process single fid file...” and select the fid file in the spectrum of interest. All processing steps are stored as CSV files and can be viewed by **Spectra View**. It is advisable to check the processed spectrum for some spots with **Spectra View** to confirm that the spectra processing is as expected. One can view the file *SpectraProcessing.xml* to check the numerical result from the different processing steps.

The generated files are:

- xy01_RawSpectrum.csv – Raw spectrum
- xy02_Quartile1Spectrum.csv – Baseline of the raw spectrum
- xy03_BaselineCorrSpectrum.csv – Corrected raw spectrum
- xy04_TransformSpectrumNormMZ.csv – SMST of the corrected raw spectrum
- xy05_TransformSpectrumNormMZBaselineProc.csv – SMST with cutoff
- xy06_TransformNormMZNormInt.csv – SMST with cutoff and normalized intensity from 0 – 1
- xy07_TransformSpectrum.csv – SMST with cutoff and the intensities corresponding masses
- xy08_InvertedTransformSpectrum.csv – Retransformed SMST spectrum
- xy09_GentleSmoothingSpectrum.csv – Spectrum smoothed with Savitzky-Golay smoothing filter
- xy10_DerivativeSpectrum.csv – Derivative of smoothed spectrum
- xy11_SpectrumNoise.csv – Spectrum noise with removed peaks
- xy12_DerivativeNoise.csv – Derivative of noise spectrum

- xy13_PeakNoise.csv – Peak noise indication spectrum
- xy14_ValleyIndication.csv – Valley indication of smoothed spectrum
- xy15_BaselineSmooth.csv – Baseline of smoothed spectrum
- xy16_RecalibratedSpectrum.csv – Recalibrated spectrum
- PeakList.csv – Peak list before recalibration
- PeakListPost.csv – Peak list after recalibration
- SpectraProcessing.xml – The result of the spectra processing

Process spectra...

To process spectra in a top folder, click the button “Process spectra...” and select the top folder.

During this process a xy.dat file and a SpectraProcessing.xml file are created in each spectrum folder.

The progress bar will indicate how much of the spectra that have been processed.

Spectra Normalization

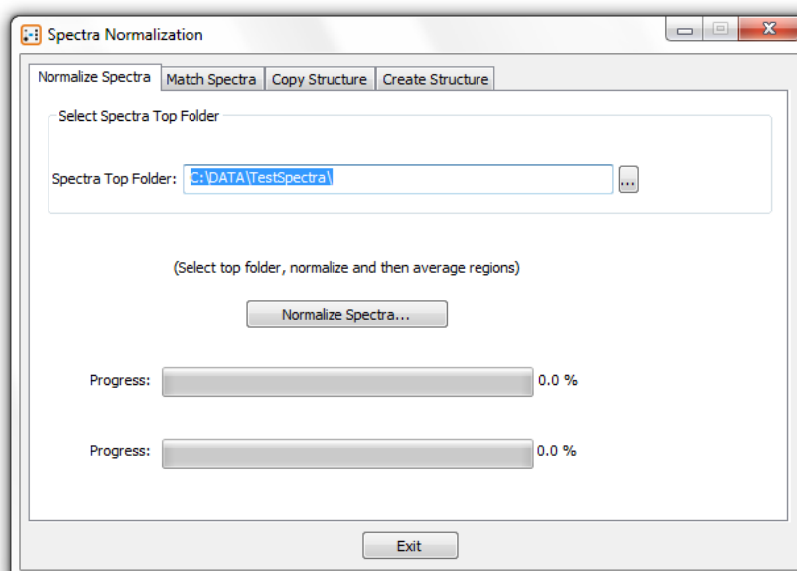
Spectra Normalization is a program that:

- Normalize spectra with different methods.
- Makes average spectra from ROI and/or tissue experiments.
- Matches normalized spectra to later defined ROI.
- Copies spectra structures when different normalization method is performed on same experiment.
- Creates spectra structures from spectra list from regions.

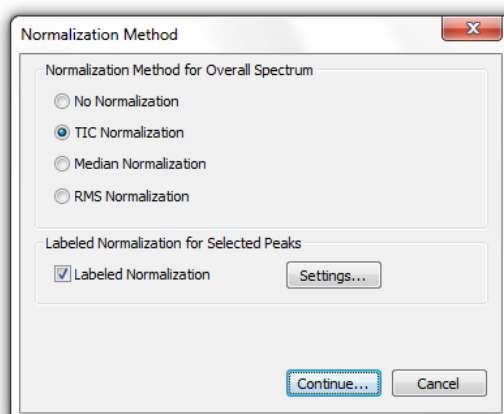


Before running this program, run Spectra Processing to create <xy.dat>.

Normalize Spectra



After the spectra processing with Spectra Processing are performed, normalization and creation of average spectra is done with Spectra Normalization – Normalize Spectra. When the normalization is performed the intensities are restored in respect to the normalization method for each spectrum. Therefore must the experiments from the control tissue and the experiment tissue be normalized at the same time. The spectra top folder must contain spectra folders for control tissue and experimental tissue. When the button “Normalize Spectra...” is pressed the following window appears:



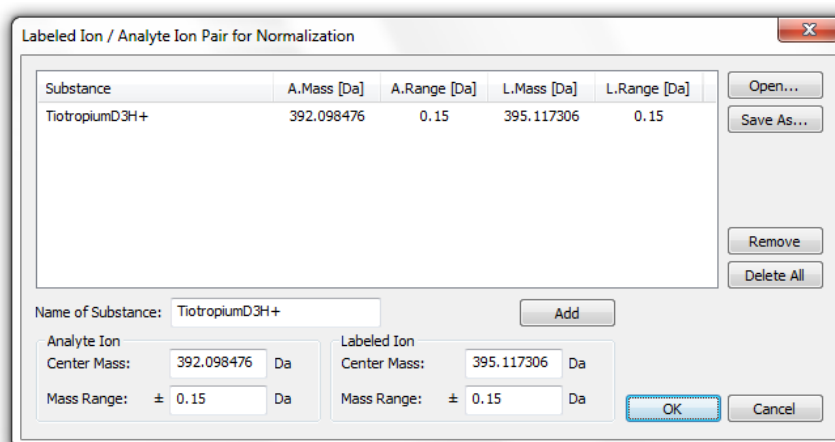
The normalization methods for the overall spectrum are:

- No Normalization
- TIC Normalization
- Median Normalization
- RMS Normalization

Select one of the normalization methods by clicking one of the radio buttons. The default selection is TIC Normalization. After the normalization two new folders are created in the spectra top folder: <AVG> and <StDev>. The folder <AVG> contains average spectra and <StDev> the standard deviation spectra.

Labeled normalization method:

If a labeled substance is added during the tissue preparation one can normalize a peak in the spectrum against this labeled substance peak. Up to 10 labeled substances can be labeled normalized in the same spectrum. To add a list of labeled substances click the button “settings...” and the following dialog appears:



The interface is more or less the same as in the Recalibration dialog in the program Spectra Processing. It is possible to open a labeled ion file that contains the calibrant needed to perform the recalibration. The file extension for the labeled ion file is “*.par”. To create a new labeled ion list just add the name of the substance in the “Name of Substance” edit window, add the analyte ion center

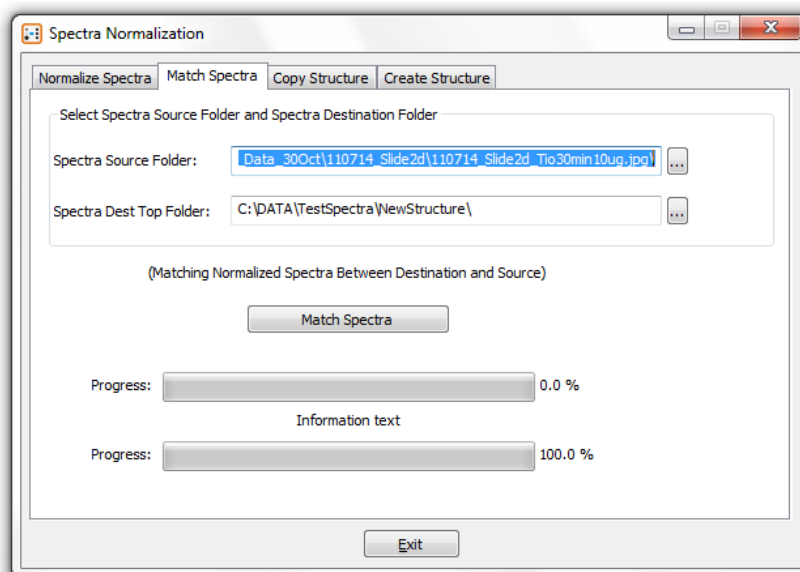
mass in Dalton and the mass range in Dalton. Also add the labeled ion center mass in Dalton and the mass range in Dalton. Push the “Add” button to add the labeled ion in the list. It is possible to edit the list in two ways, delete all substances by clicking the button “Delete All” or delete one substance by first select one and then click the button “Remove”. When the list is created save the list with a unique name by pushing the “Save As...” button.

Do not forget to check the labeled normalization button if a labeled normalization shall be performed. If no other overall normalization is needed for the rest of the spectra like TIC Normalization, just click “No Normalization”.

Click on the button “Continue...” to perform the normalization and average spectra creation.

Match Spectra

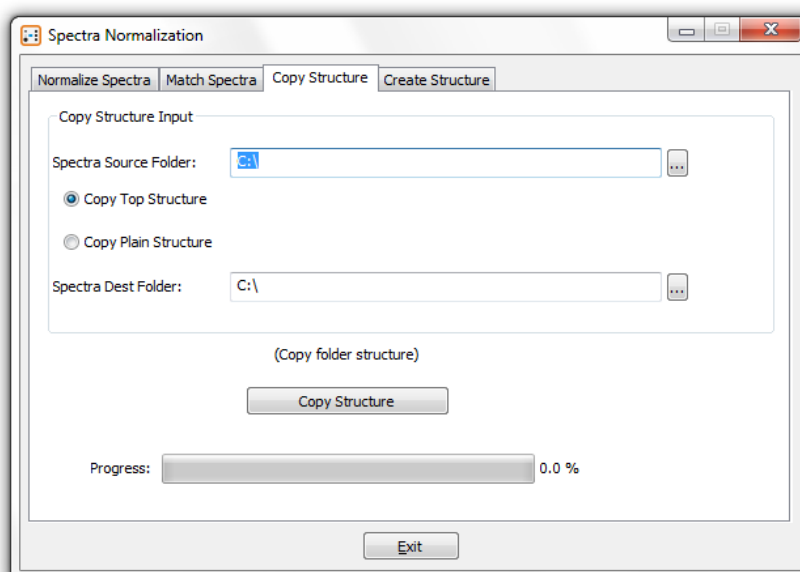
If regions of interest (ROI) have been defined and the spectra are located in a new top folder contained with the different ROI spectra folders one need to match these new spectra with the normalized spectra that has been performed in the first step. This is done by first selecting the spectra source folder that contains the e.g. control tissue spectra. Second select the spectra destination top folder that contains the ROI.



Continue the spectra matching by clicking the button “Match Spectra”. The normalized spectra are copied to the ROI spectra folders and an average spectrum is calculated for each ROI.

Copy Structure

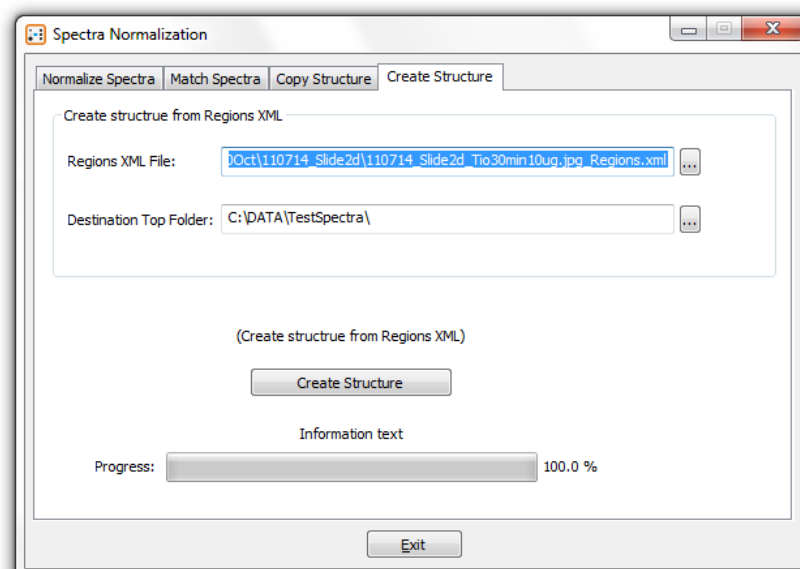
If data processing with several different normalization methods shall be done it is possible to copy the spectra structure.



It is possible to copy a whole top structure or just a plain structure as a single spectrum folder. The information that is copied is just folder structure, not the underlying data.

Create Structure

To create folder structures containing ROI and their spectra, select first the spectra list from regions xml file (created by Bruker Daltonics flexImaging™). Select also the destination top folder where the ROI folders will be created.



Click on the button Create Structure to continue. To fill the created folder structures with normalized spectra, go back to the tab Match Spectra and continue.

Quantitation Tool

Quantitation Tool is a program that:

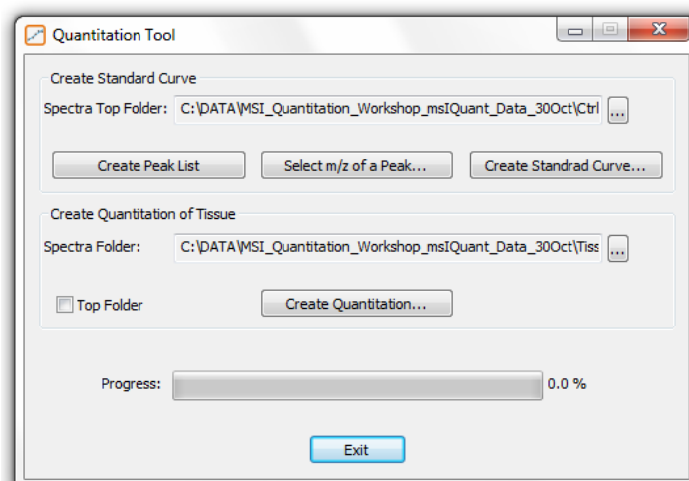
- Performs a concentration study and creates a standard curve for quantitation.
- Performs quantitation of tissue/ROI and creates an image xml file with substance concentration for each pixel.



After normalization and creation of average spectra the next step is to perform standard curve and quantitation of experiment tissue.

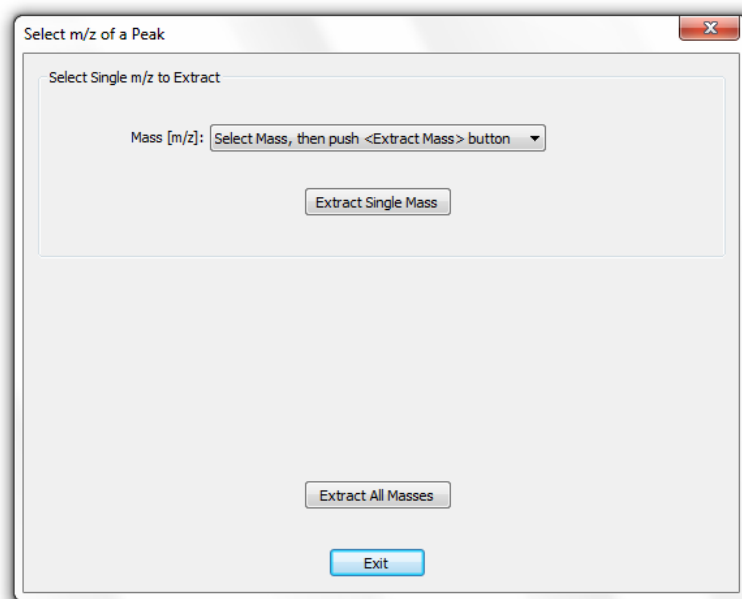
Before running this program, run Spectra Normalization to create average spectra.

Create standard curve



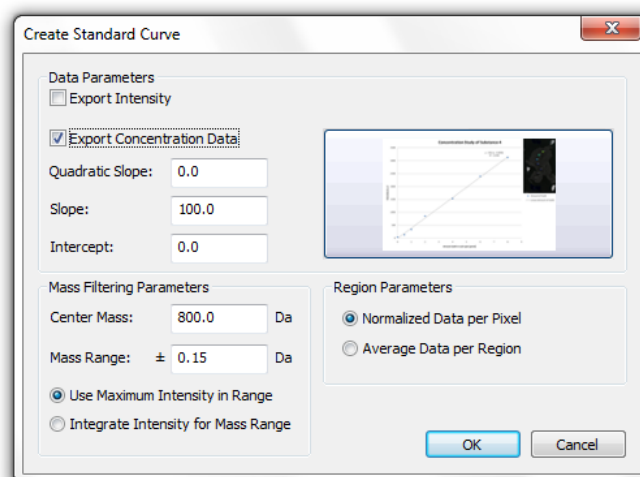
The first step in creation of a standard curve is to select the top folder that contains the regions of interest (ROI) of the different spotted concentrations. When this is done, push the “Create Peak List” button. This action will create peak lists of each average spectrum.

The second step is to select the peak of interest by pushing the button “Select m/z of a Peak...”. The following window appears:



Click on the combo box button and a list of all peaks and their centroid mass and average peak height will appear. Select the peak mass of interest and click on the button “Extract Single Mass”. After peak extraction click the “Exit” button to close the window.

The third step is to create a standard curve based on the mono isotopic peak from the substance that is investigated. Continue to click the button “Create Standard Curve...” and the dialog Create Standard Curve appears:



Enable the checkbox “Export Concentration Data” and then click on the big button with a graph bitmap on and the next dialog Concentration Study will appear.

Create Standard Curve

Select Peak List File

Peak List File:

Mass [m/z]: 392.0997 Da (Double Click on Region to Select a New Amount)

Region	Amount	Peak Height	Amount/mg
0.000pmol	0.0	0.0	0.0
0.080pmol	0.08	129.28	2.691565
0.160pmol	0.16	190.78	5.383129
0.320pmol	0.32	719.97	10.766259
0.640pmol	0.64	1683.28	21.532518
1.300pmol	1.3	5705.45	43.737926
2.600pmol	2.6	11470.37	87.475853
5.000pmol	5.0	22971.45	168.222794

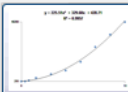
Select Type for Slope/Intercept Calculation

☐ Use Amount
 ☐ Use Amount/mm³
☒ Use Amount/mg

Spot Diameter: mm
 Tissue Thickness: µm
 Tissue Density: g/cm³
 Tissue Volume: mm³
 Tissue Mass: mg

Slope: 138.969269
 Intercept: -544.071665
 R²: 0.997651

☐ Polynomial Regression
☐ Zero Intercept



To find the extracted peak from the second step go to the top folder and the into the <PeakList> folder, there is the extracted peak file (e.g. the file <Peak_281.2034.csv>). To make it easy to find the file the mass of the centroid peak is attached to the file name. In the list box the regions from the concentration spots appear. To edit the amount of added substance double click on the region name and edit the correct amount in the edit window that appears and enter the return key. If one spots should be an outlier single click on the region and click on the button "Remove". The slope, intercept and R² for the standard curve are updated simultaneously. There are three different selections that can be made to calculate the concentrations:

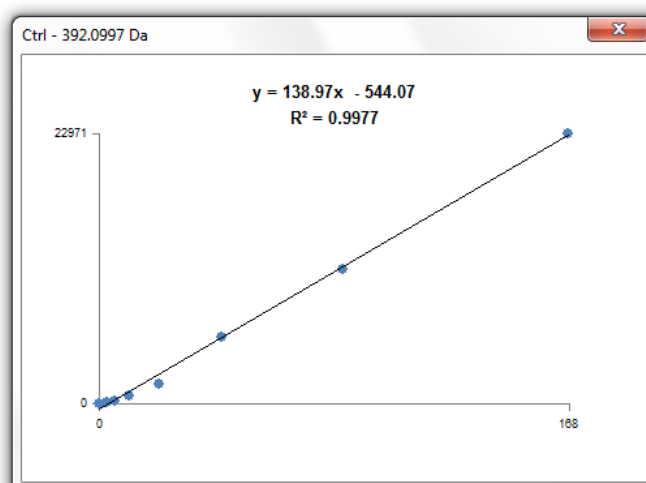
- The amount of substance alone
- The amount per mm³ tissue
- The amount per mg tissue

Select the concentration option in the group box "Select Type for Slope/Intercept Calculation". Depending on the selection, measure the average spot diameter, tissue thickness and tissue density. For convenience the selected tissue volume and tissue mass will be calculated.

Tip! If the whole spot is selected and the pixels in the spot are extracted, count the pixels and multiply them with the x and y resolution to calculate the area. The diameter from the average

area of the spots can be calculated by the formula $d = \sqrt{\frac{4A_{avg}}{\pi}}$ and is easier then predict the diameter by measuring the picture.

To view the standard curve, click on the button with bitmap picture of a graph. Below is an example of how it looks like.



To force the standard curve to go through origin, enable the check box “Zero Intercept”. There is also another option to form a 2nd order polynomial by enable the check box “Polynomial Regression”. This might be handy if low amounts of the substance are spotted and ion suppression is present. If this option is selected the “Zero Intercept” cannot be selected in this version of the program.

When all settings are done click on the “OK” button and the settings will be transferred to the previous dialog “Export Concentration Data”.

Create Standard Curve

Data Parameters

☐ Export Intensity

☒ Export Concentration Data

Quadratic Slope: 0.0

Slope: 138.969269

Intercept: -544.071665

Mass Filtering Parameters

Center Mass: 392.0997 Da

Mass Range: ± 0.15 Da

☒ Use Maximum Intensity in Range

☐ Integrate Intensity for Mass Range

Region Parameters

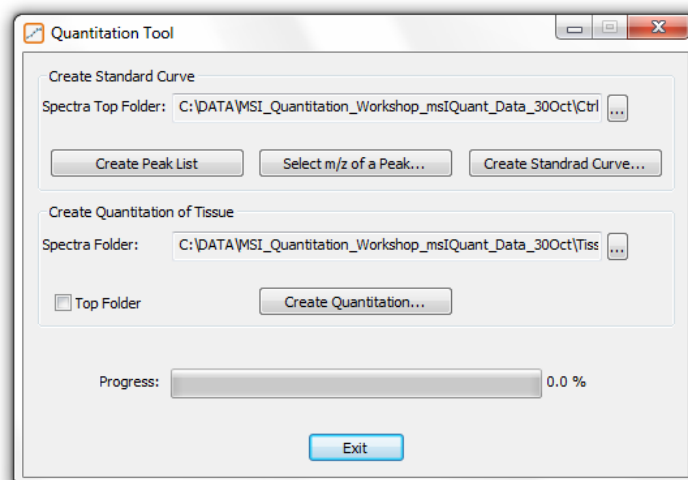
☒ Normalized Data per Pixel

☐ Average Data per Region

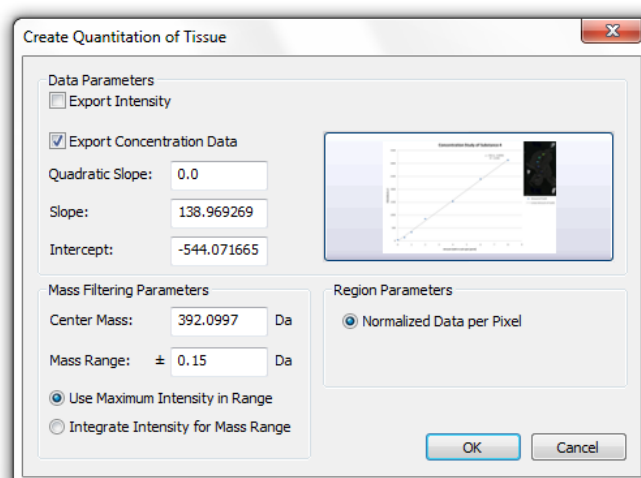
OK Cancel

It is now possible to generate an image xml file for the concentration spots. A selection that influences the image xml file is the “Region Parameters”. If a uniform concentration is required per spot, select “Average Data per Region”. Otherwise leave as is. Click the “OK” button to generate the image xml file.

Create quantitation of tissue



When quantitation is made on experimental tissue, it can be done to the entire tissue or on the ROI from the experimental tissue. Select spectra folder for the experimental tissue if quantitation of the whole tissue shall be done. However, if quantitation of the ROI of the experiment the tissue shall be done, select the spectra top folder and enable the checkbox “Top Folder”. Then press the button “Create Quantitation ...” and the following dialog Create Quantitation of Tissue appears:



The default settings are the same as in the Create standard Curve dialog. Click the “OK” button to generate the image xml file(s).

For information! The naming of the generated image xml file is “ExpConc” + foldername + center mass + mass range + H or A + P or R. H is when the peak intensity has been used and A when the peak area has been used. P is when pixel by pixel is calculated and R when a whole region is calculated.

XML Evaluation

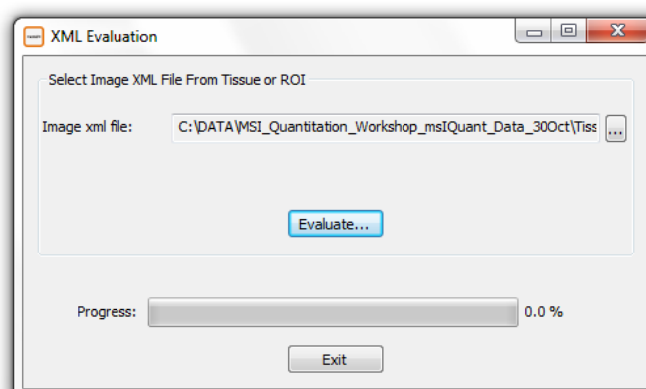
XML Evaluation is a program that performs calculation of the generated xml file from the program Quantitation Tool. The information and calculation of an xml file are:

- Number of pixels
- Average quantified value
- Standard deviation (STD) of the quantified value
- Relative standard deviation (RSD) of the quantified value
- Min value of the quantified value
- Max value of the quantified value
- Pixel deviation analysis (PDA)
- Pixel ratio calculation (PRC)

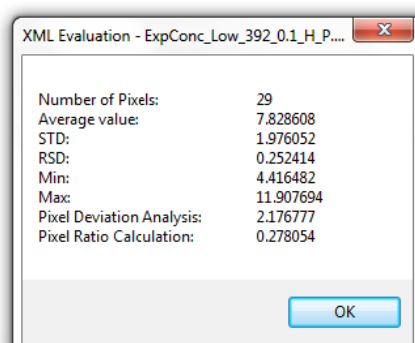


Before running this program, run Quantitation Tool to create image xml file(s).

Perform xml evaluation



Select an image xml file and click on the button “Evaluate...” to evaluate the file. The next dialog box will appear:



The information in the dialog is automatically copied to the clipboard to easily past into e.g. Microsoft Excel. Below is an example from Excel when the evaluation is pasted:

	A	B	C	D	E	F	G	H	I	J
1	ExpConc_Cx_281_0.5_H_P.xml	287	113.7996	65.42072	0.574877	0	479.3134	66.25685	0.582224	
2										
3										

Pixel analysis

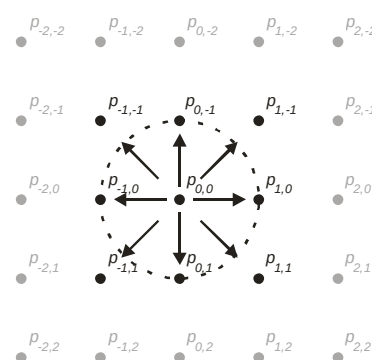
The program makes analysis of neighbor pixels how much they differ from each other with by Pixel Deviation Analysis and Pixel Ratio Calculation.

Below is a description of the algorithms:

Pixel Deviation Analysis

The resolution does not matter when performing a pixel deviation analysis with quadratic pattern since the relative distance is always the same.

The algorithm works in the way that the distance to all eight neighbors is the same and is represented by the dotted circle around the central pixel.



Each pixel is named with x and y index $p_{x,y}$. For each pixel there is an intensity also named with x and y index $I_{x,y}$. The abbreviation of Pixel Deviation Analysis is for short PDA.

Differences for each neighbor pixel are calculated:

$$D_{1,0} = I_{1,0} - I_{0,0}; \quad D_{0,1} = I_{0,1} - I_{0,0}; \quad D_{-1,0} = I_{-1,0} - I_{0,0}; \quad D_{0,-1} = I_{0,-1} - I_{0,0}$$

...except for the corner pixels where the differences are divided by the square root of 2.

$$D_{1,1} = (I_{1,1} - I_{0,0}) / \sqrt{2}; \quad D_{-1,1} = (I_{-1,1} - I_{0,0}) / \sqrt{2}; \quad D_{-1,-1} = (I_{-1,-1} - I_{0,0}) / \sqrt{2}; \quad D_{1,-1} = (I_{1,-1} - I_{0,0}) / \sqrt{2};$$

The root mean square (RMS) is then calculated of the eight differences (the PDA is equal to the RMS).

$$PDA = RMS = \sqrt{\frac{1}{8} (D_{1,0}^2 + D_{0,1}^2 + D_{-1,0}^2 + D_{0,-1}^2 + D_{1,1}^2 + D_{-1,1}^2 + D_{-1,-1}^2 + D_{1,-1}^2)}$$

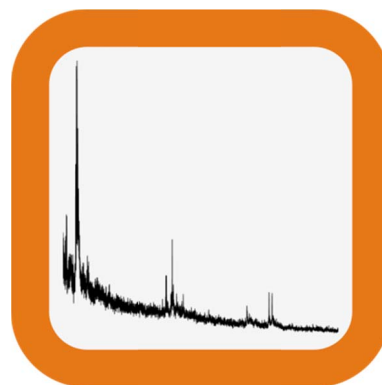
Pixel Ratio Calculation

The Pixel Ratio of a region of interest (ROI) is the average Intensity from the pixels divided by the average PDA of the pixels.

Spectra View

Spectra View is a program that displays mass spectra files. It is capable to display following files:

- star.dat mass spectra files.
- star.csv mass spectra files.
- fid raw mass spectrum files that is created by Bruker Daltonics flexControl™.

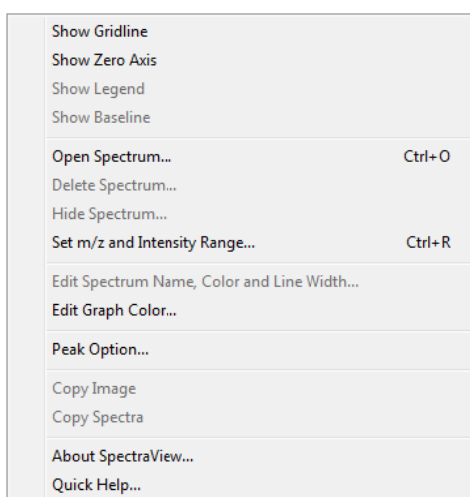


The format of the dat and csv files are text files where the first line can be a header that contains information like “m/z” and “Intensity”. The file must contain only two columns where the first column is the mass and the second column is the intensity. The decimal separation must be a dot ‘.’ (character value 0x2e) and the column separation character can be a tab (character value 0x09), space ‘ ’ (character value 0x20) or a comma ‘,’ (character value 0x2c). The line must end with carriage return (character value 0x0d) and line feed (character value 0x0a).

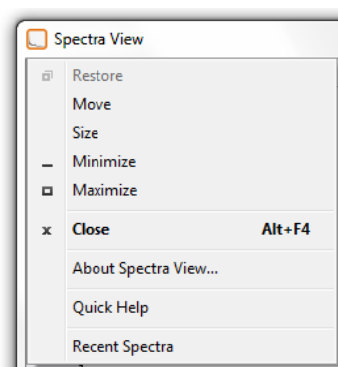
How to open mass spectrum files

Since *.dat files are associated with Spectra View it will start Spectra View and open the file directly when double clicking on it in explorer. Normally *.csv files are associated with Windows Excel but to open it from explorer, right click on the *.csv mass spectrum file and click on “Send To” and select “Spectra View” that is in the menu. To open a fid mass spectrum raw file in explorer, right click on the fid file and select “Open” in the menu and select Spectra View in the Programs list.

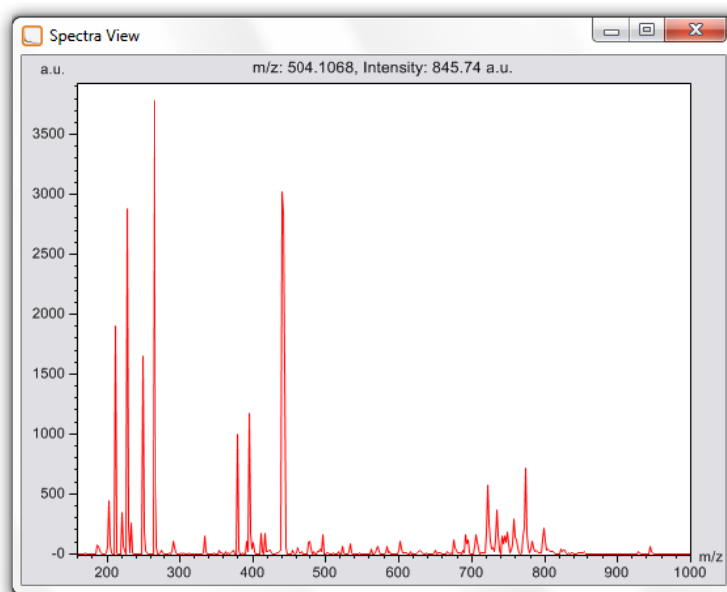
It is also possible to start Spectra View and right click on the window and the following popup menu appears:



Select “Open Spectrum...” in the popup menu or just press <Ctrl+O> to open a mass spectrum. It is also possible to open several spectra with multiple selections. Once a spectrum is opened it is possible to open one or several more spectra. If spectra have been edited it is possible to reopen them again by selecting “Recent Spectra” in the system menu.



Below is an example of how a mass spectrum is displayed:



How to zoom in and out and how to move spectra

During the zoom operation different cursors appear and their specific zoom operation can take action:



When the *Zoom in m/z* cursor appears it is possible to zoom in and out the *m/z* axis by moving the scroll wheel on the mouse back and forth.



The *Zoom in intensity* cursor appears by pressing <Ctrl> key on the keyboard. It is then possible to zoom in and out the intensity axis by moving the scroll wheel on the mouse back and forth.



If the *Zoom in m/z* or *intensity* cursors appears and the spectrum mass spectrum is zoomed, it is possible to move the spectrum in both *m/z* and intensity direction by holding down the left mouse button and move the mouse on the spectrum area. Once the spectrum starts to move, the *Hand* cursor appears.



The *Zoom in* cursor appears by pressing the <Alt> key on the keyboard. It is then possible to zoom in the spectrum by selecting the zoom area with the mouse.



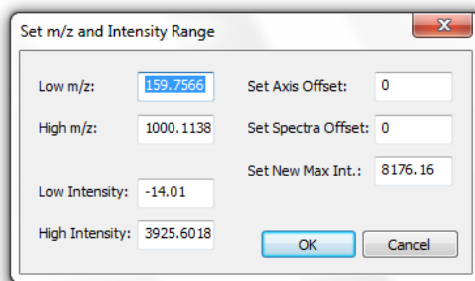
The *Mass axis arrow* cursor appears when holding the cursor over the m/z axis. Double click with the left mouse button on the m/z axis to rescale to the full m/z range. If the mass spectrum is zoomed it is possible to move the spectrum in m/z direction by holding down the left mouse button and move the mouse on the m/z axis.



The *Intensity axis arrow* cursor appears when holding the cursor over the intensity axis. Double click with the left mouse button on the intensity axis to rescale to the full intensity range. If the mass spectrum is zoomed it is possible to move the spectrum in intensity direction by holding down the left mouse button and move the mouse on the intensity axis.

How to select a specific m/z and intensity range or set axis and spectra offset

To set a specific m/z and intensity range, right click the right mouse button and click “Set m/z and intensity range...” in the popup menu or just press **<Ctrl+R>**.



Set the values to get specific range of m/z and intensity and click the “OK” button.

If the spectrum is close to the zero intensity it is possible to set a negative axis offset value to be able to “lift up” the spectrum.

If several spectra are displayed it is possible to separate them by setting the spectra offset.

If a peak is investigated and it is the highest in the whole spectrum, it is possible to set a new maximum intensity to zoom out the spectrum.

How to measure m/z and intensity

The information about m/z and intensity is shown all the time when the mouse is moved over the graph area. It is also possible to measure the m/z and intensity difference:



The *Measure* cursor appears when pressing **<Shift>** key on the keyboard and is used for measuring $\Delta m/z$ and Δ intensity. Move the cursor to a specific peak, press down the left mouse button and move the cursor to a new peak and release the mouse button. A message box appears with the information about the $\Delta m/z$ and Δ intensity. The information is automatically copied to the clipboard to be able to paste it directly to e.g. Microsoft Excel.

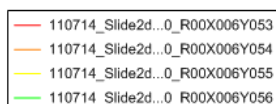
Display options

The display options are; Show Gridline, Show Zero Axis, Show Legend, and Show baseline. They can be all set in the popup menu.

When Show Gridline is set it displays the major gridline for both intensity and m/z .

When Show Zero Axis is set it displays the zero intensity axis in the spectrum.

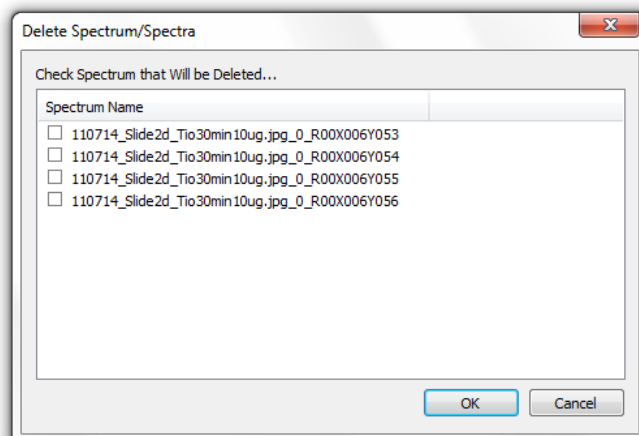
When Show Legend is set it displays the legend of the spectra. The legend can be moved in the window but is set in default position when the window is resized. Below is an example how the legend can look like.



When Show baseline is set the baseline is displayed in the same color and width as its spectrum but with a dashed line.

How to delete or hide spectra

When spectra are opened and if one wants to delete a spectrum or spectra; select the “Delete Spectrum...” from the popup menu.



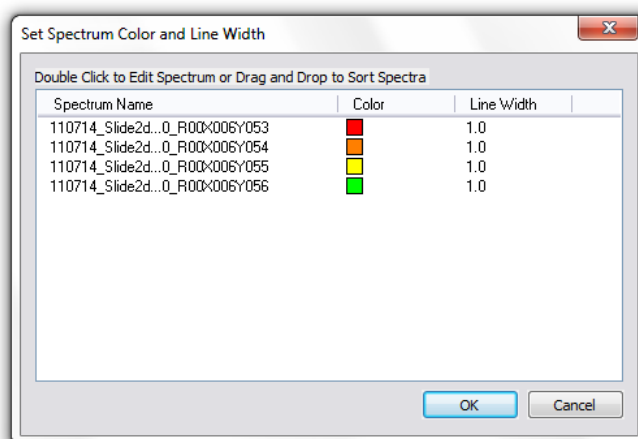
Check the spectrum that is going to be removed and click “OK”.

If one wants to hide one or more spectra; select the “Hide Spectrum...” from the popup menu. Check the spectrum that is going to be hid and click “OK”.

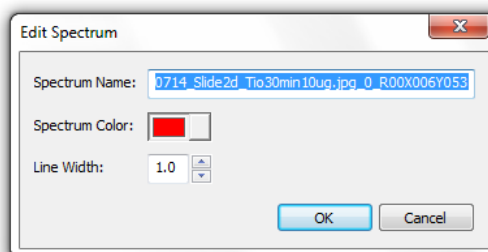
How to edit spectra names, colors, line widths and spectra order

To change order of the spectra, click on an item and drag and drop the item to the new position in the list.

To change the spectra names, colors, line widths and spectra order; select the “Edit Spectrum Name, Color and Line Width...” in the popup menu.



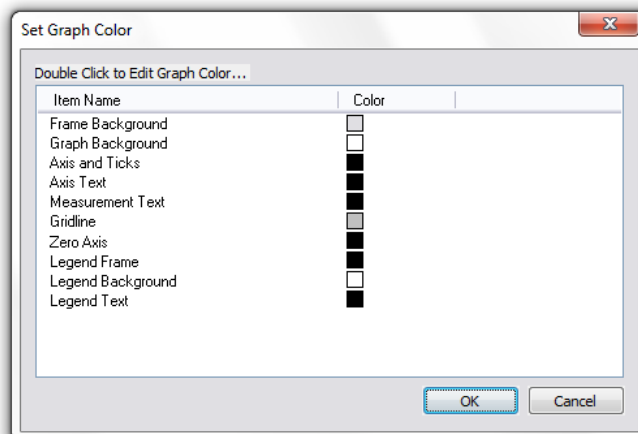
To change the name, color and line width of a spectrum, double click on the item and the following dialog window is shown:



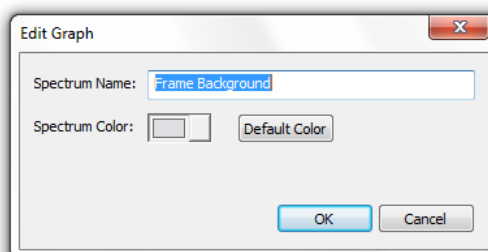
Edit the spectrum and click “OK”.

How to edit the graph colors

To customize the graph colors, select the “Edit Graph Color...” in the popup menu.



To change a color, double click on the item and select a new color:



How to copy the image

To copy the graph image to the clipboard, select “Copy Image” in the popup menu and paste it directly to e.g. Microsoft PowerPoint.

How to copy the spectra within a specific m/z range

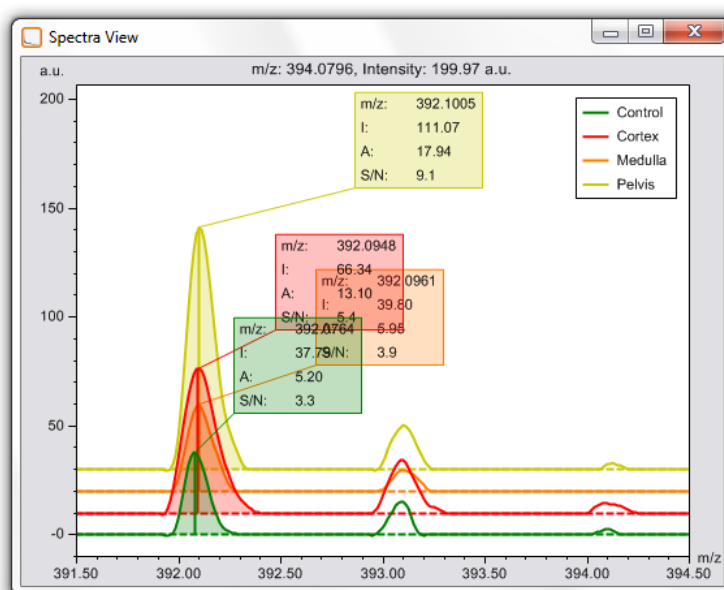
To copy the spectra in the window to the clipboard, select “Copy Spectra” in the popup menu and paste it directly to e.g. Microsoft Excel.

How to select specific spectra peaks

Zoom in and display the peaks of interest.



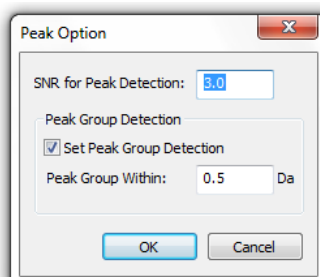
To select the *Peak* cursor, click once on the <Tab> key. Then click on the left side of the peak and the graph will display information of the centroid m/z , centroid intensity, the peak area and the signal to noise ratio.



The information about the selected peaks is automatically copied to the clipboard to be pasted to e.g. Microsoft Excel.

How to set peak picking parameters

To change parameters for peak picking, select the “Peak Option...” in the popup menu.

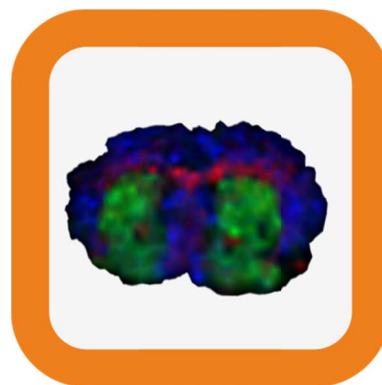


Edit the signal to noise ratio for peak selection. Edit the mass frame to be able to select a group of peaks.

Image View

Image View is a program to display an image xml file generated from the Quantitation Tool.

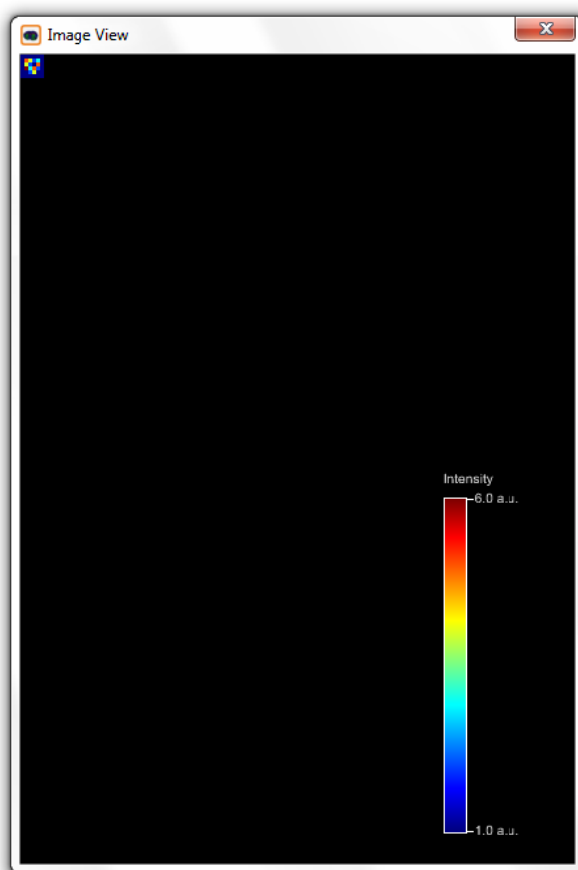
The Image View is in its present state more or less a prototype of displaying images. The goals with this software were to investigate rendering principle, interpolation methods, color scale implementation and overlay of up to three different images. The image xml file is compatible with Bruker Daltonics flexImaging™ and can imported as well.



The rendering technology is called Silk-Screen rendering. The bitmap image that is displayed is transferred from the original image that is vectorized.

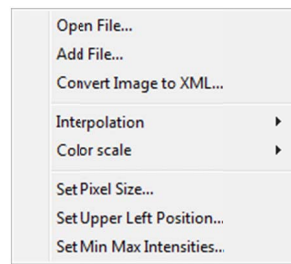
How to open image xml files

When the Image View has started a small image with 4x4 pixels is viewed. The image comes from the article: Bicubic interpolation from Wikipedia (http://en.wikipedia.org/wiki/Bicubic_interpolation).



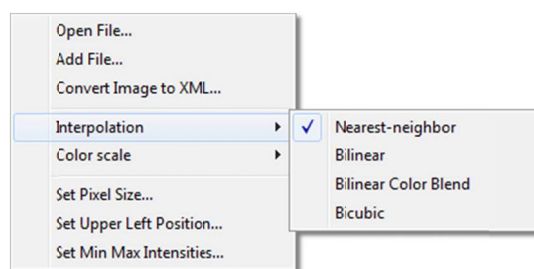
It is possible to open an image xml file in several different ways. If the program is already open one can drag and drop an image xml file or open the image xml file from a popup menu by right click the window. If the image xml file is listed in the explorer one can open the file by right clicking on the file and further click on "Send To".

Below is the popup menu displayed:



How to select different interpolation methods

The default interpolation method of an image is nearest-neighbor. To change the method, click on the Interpolation and select one of four interpolation methods.



Below is the four different interpolation methods displayed:



Nearest-neighbor interpolation



Bilinear interpolation



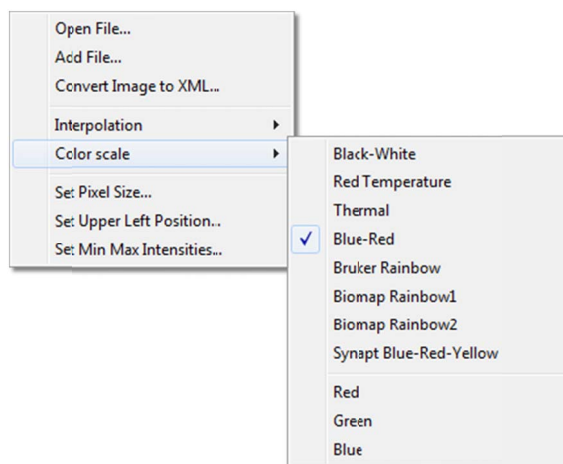
Bilinear Color Blend interpolation



Bicubic interpolation

How to change color scale

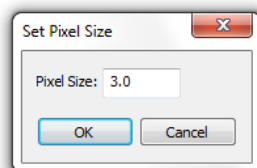
There are eleven different color scales to express the intensities for one m/z channel, all with linear scale.



It is possible to view up to three different m/z channels but only with red, green and blue color scales.

How to change the pixel size

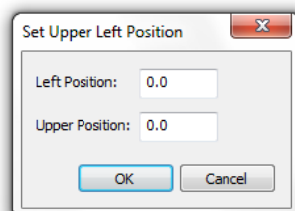
Select "Set Pixel Size..." in the popup menu to change the pixel size. It is possible set a floating point as a pixel size since the image rendering is vectorized.



In present version of the software a pixel is squared so it is not possible to set different values of x and y direction.

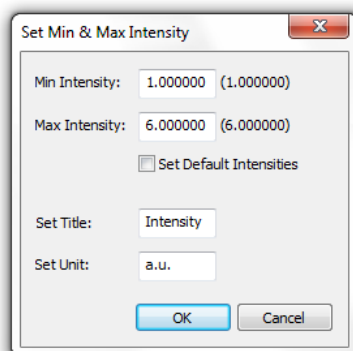
How to change the upper left position of an image

Select "Set Upper Left Position..." in the popup menu to change the upper left position. It is possible to set a floating point of the position since the image is vectorized.



How to change the parameters of the color scale, title and unit

To change the value of the min and max intensity, select the “Set Min & Max Intensity” in the popup menu. To return to the default intensities, check the “Set Default Intensities” and click on OK button.



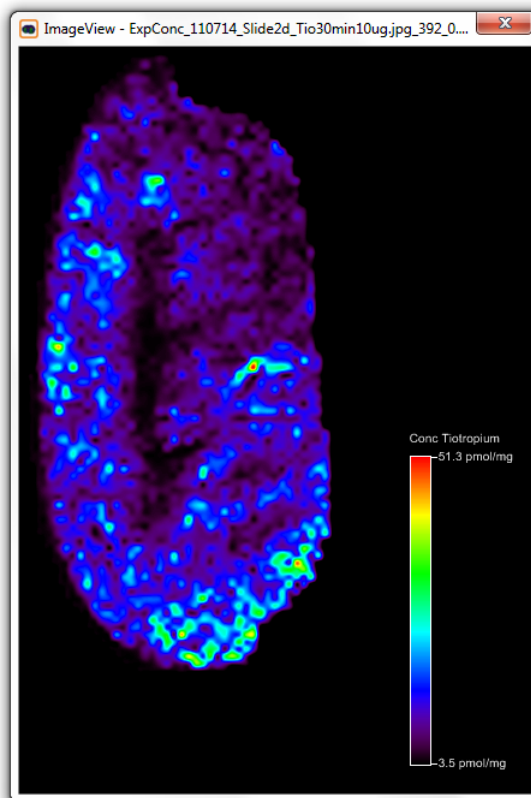
It is also possible to change the title and the unit of the color scale.

How to save an image generated by Image View

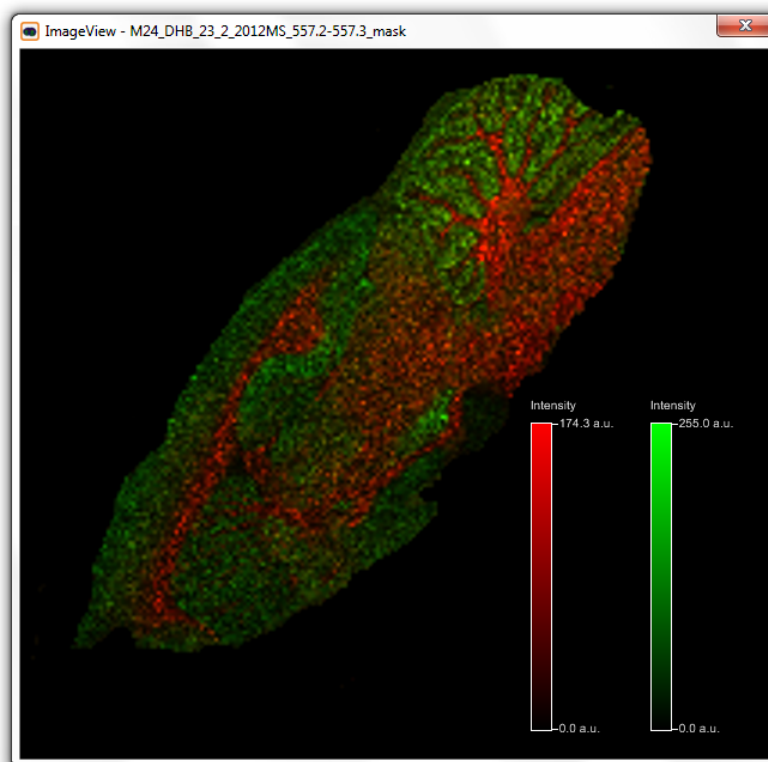
The present version of mslQuant has no export possibility of Image View. The best way to save an image is to have the focus on Image View and to press <Alt> + <PrtScn> to copy the image to the clipboard. Then paste the image to the software where you want to show the image.

Examples of displaying a single or multiple m/z channels

Below are two examples of images. The first image displays a single m/z channel.

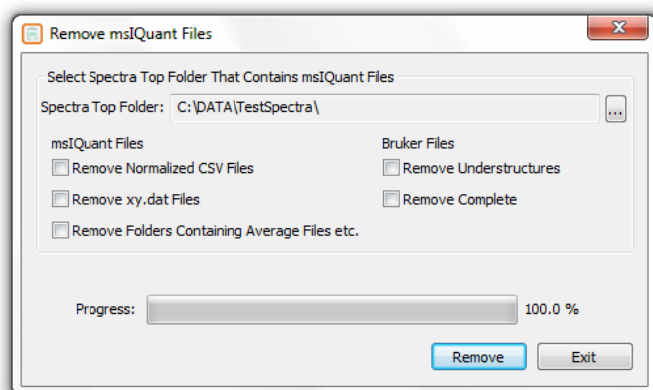


The second image displays two different m/z channels and how they are distributed in the tissue.



Remove mslQuant Files

Remove mslQuant Files is a program that removes mslQuant generated files when they are not needed any longer. It is also possible to remove redundant data when it is not needed any longer to save disk space.



Remove Spectra Processing files

Spectra Processing generates xy.dat files in every spectrum folder. To remove these files when they are not needed any longer, select the spectra top folder where they are contained, check “Remove xy.dat Files” and click on the “Remove” button. The progress bar tells when the erase operation is complete.

Spectra Normalization generates normalized CSV files. To remove these files when they are not needed any longer, select the spectra top folder where they are contained, check “Remove Normalized CSV Files” and click on the “Remove” button. The progress bar tells when the erase operation is complete.

Spectra Normalization also generates the folders; “AVG”, “StDev” and “PeakList”. To remove these folders with their content, select the spectra top folder where they are contained, check “Remove Folders Containing Average Files etc.” and click on the “Remove” button. The progress bar tells when the erase operation is complete.

Remove Bruker Files

When ROI spectra are copied all information including raw files etc are copied as well. This information is not needed when running mslQuant, only the empty spectrum folders. To reduce the disk space it is possible to remove this understructure. To do this, select the spectra top folder where they are contained, check “Remove Understructures” and click on the “Remove” button. The progress bar tells when the erase operation is complete.

When a copy of a whole experiment shall be erased it is possible to do this by, selecting the spectra top folder where the experiment is contained, check “Remove Complete” and click on the “Remove” button. The progress bar tells when the erase operation is complete.

Appendix – Abbreviations

Abbreviation	Explanation
CSV	Comma-Separated Values
IMS	Imaging Mass Spectrometry
m/z	Mass to charge ratio
MALDI	Matrix-Assisted Laser Desorption/Ionization
MS	Mass Spectrometry
R^2	Coefficient of determination
PDA	Pixel Deviation analysis
PRC	Pixel Ratio Calculation
RMS	Root Mean Square
ROI	Region of Interest
RSD	Relative Standard Deviation
SD	Standard Deviation
SMST	Sorted Mass Spectrum Transform
SMQ1	Simple Moving First Quartile
SNR	Signal to Noise Ratio
TIC	Total Ion Current
TOF	Time of Flight
xml	eXtensible Markup Language

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